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Synthesis and Characterization of Molecularly Imprinted Polymer Caffeine Using Acrylamide as Monomer

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Abstract—Objectives: Molecularly Imprinted Polymer (MIP) is a selective polymer that can bind target molecules, is made with a ratio 1:4:20 (mmol) between caffeine, acrylamide, and ethylene glycol dimethacrylate. The ratio will form a polymer that will be released again so that it can selectively recapture the template. Method: The synthesis method of MIP and NIP was using the bulk method with direct heating at 60ºC for 8 hours. Molecular release using extraction and sonication method with chloroform solvent resulted in 20 times the recovery until the value released below the selection limit of 1.173 ppm. To find out the effectiveness of polymers in capturing the template, a batch method was repeated on theophylline with were the same xanthine derivates. Results and Discussion: Polymers against caffeine are more selective than theophylline which is 76.55% and theophylline is only 1.31%. Fourier Transform Infra-Red (FTIR) was using characterization, there is a functional group of differences between the FTIR spectrum of caffeine, MIP with caffeine, MIP without caffeine and NIP. Conclusion: The resulting adsorb of MIP has a selective than NIP with value Imprinting Factor (IF) 1.26. Of the sample testing obtained MISPE has a selective than NISPE with the percentage of concentration analgesic drug sample 82.01% and energy drink sample 84.01%.

Keywords: MIP, caffeine, acrylamide, FTIR, MISPE

I. INTRODUCTION

Activities or activities that cause fatigue and drowsiness which means that can be handled by consuming energy drinks containing caffeine as an effort to increase totality in a job [1]. According to SNI 01-7152-2006 states that the content of coffee will have a beneficial effect if the caffeine level is between 150 mg/day and 50 mg/serving, while according to the FDA (Food Drug Administration) the allowed dose of caffeine is 200 mg/day [2].

In general, caffeine can be analyzed by the HPLC or HPLC [3-5] method and UV-Vis spectrophotometry [2, 6, 7]. However, this method will be less sensitive if done on a complicated sample matrix because other compounds can intervene during the process analysis. One method of sample preparation for caffeine determination is solid-phase extraction and liquid phase extraction. In analyte separation, liquid-phase extraction is very easy to do. However, it still has many disadvantages such as the limitation of the solvent used for a sample in a sufficient number of solvents and the difficulty of separating the solvent because the recovery results often form an emulsion mixture. These weaknesses can be overcome by using the solid phase extraction method because they have time efficiency, better recovery results and more efficient use of solvents [8].

Separation technique using MISPE or printed polymer of solid-phase extraction molecule which is currently being developed [9]. MIP or printed polymer is sorbent in the form of material in the use of MISPE, which is a new technique that has the ability such as enzymes to selectively capture substrate [10]. Besides its ease of use to form a polymer made from mold molecules, functional monomers, crosslinkers, and initiators, MIP has high selectivity to separate analytes from target molecules [11]. Thus, a separation technique using MIP is used to analyze caffeine with functional acrylamide monomers in caffeine-containing samples. So that the synthesis and characterization can be done with this technique [12].

II. MATERIAL AND METHOD

A. Synthesis of MIP and NIP

A total of 194.19 mg of caffeine was dissolved in 2 mL chloroform in a special bottle then vortexed for 15 minutes to ensure that the caffeine was dissolved. 284.32 mg of acrylamide were put into another reaction bottle (which had been dissolved in 3 mL chloroform) for introduction in the 15-minute vortexed template molecule. As much as 3.8 ml of EDGMA was added then vortexed 15 minutes and 5 mL AIBN was added to the reaction bottle, vortexed for 30 minutes, then flowed nitrogen gas for 5 minutes to remove oxygen. The bottle is tightly closed and sonicated for 8 minutes. Then put into the oven temperature of 60oC for 8 hours, observed every 1 hour, then cooled in a desiccator. The results of the synthesis are crushed and then sieved in sieves number 40 and number 60, then weighed [13, 21].

Making NIP is done as a comparison with procedures and composition similar to MIP but in making NIP it is not done by adding caffeine as a template [13, 21].

B. Release of Template Molecules

A total of 1500 mg of MIP synthesis results were extracted with 10 mL chloroform, then sonicated 30 minutes, then filtered using filter paper, the extracted filtrate was collected in vials and then analyzed using UV-VIS spectrophotometry. After the filtrate is collected, the MIP is dried. Done until the final template is released perfectly.

C. MIP and NIP characterization

Caffeine, MIP with templates, MIP without templates and NIP before extraction are characterized by FTIR. Samples were carried out using KBr pellets in which the powder formed samples mixed with KBr in a ratio of 1:99 were crushed until homogeneous. Then put into a printed disk, then compressed at a pressure of 20 psi using a hydraulic press. The disc was mounted on the holder and then measured at wavenumbers 4000-400 cm^{-1} using FTIR [13, 21].

D. Adsorption and Desorption Capacity Capabilities

To determine the adsorption capacity, caffeine is dissolved using chloroform with various concentrations of 9, 11, 13, 15, 17 and 19 ppm. 20 mg of MIP without a template and NIP is included in each caffeine concentration. Incubated for 24 hours at room temperature, then filtered using filter paper. The supernatant was measured using UV-VIS Spectrophotometry to see the concentration of caffeine left in solution. A calibration curve is made between the amount adsorbed and the initial concentration of caffeine. So that it will produce Imprinting Factor (IF). To determine the desorption capacity, from the MIP and NIP adsorption capacity results, added 5 mL chloroform, then sonicated for 10 minutes. The extracted supernatant was measured using UV-VIS spectrophotometry. A curve is made between the amount that is degraded and the initial caffeine concentration [13, 21].

E. MIP Selectivity without Templates

To see the selectivity of MIP without templates using xanthine derivatives, namely theophylline. Theophylline is made at a concentration of 500 ppm, absorbance is measured, then theophylline is passed into 50 mg MIP without a template re-measured its absorbance using UV-VIS spectrophotometry [14].

F. Testing Samples Circulating on the Market

Samples were extracted using 10 mL chloroform and then sonicated for 30 minutes, then filtered. Conditioning is carried out by passing chloroform into the modified MISPE and NISPE cartridge columns. 50 mg of MIP without a template and NIP is inserted into the column then the sample is passed and the results are measured using UV-Vis Spectrophotometry. Elution with chloroform was returned and analyzed using UV-Vis spectrophotometry [15].

III. RESULTS

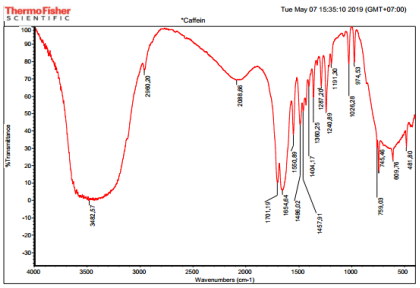

Fig. 1. The spectrum of FTIR caffeine

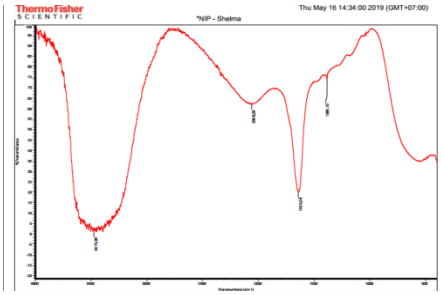

Fig. 2. NIP FTIR Spectrum

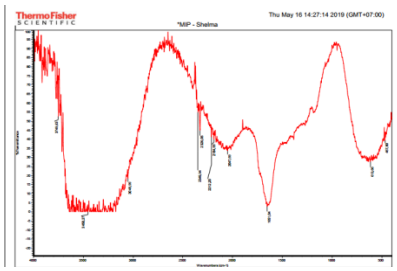

Fig. 3. MIP FTIR Spectrum before extraction (with template)

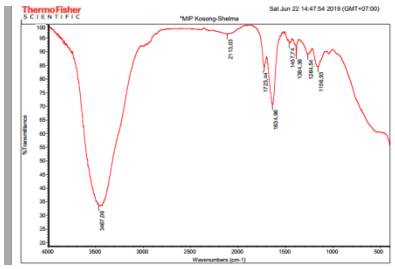

Fig. 4. MIP FTIR spectrum without template (after extraction)

IV. DISCUSSION

Synthesis of MIP and NIP is done by the method of the fraction. MIP is synthesized in a ratio of 1:4:20 (mmol) consisting of a mold molecule: functional monomer: crosslinker. Caffeine mold molecules, functional acrylamide monomers that function as binding molecular molds in polymers, ethylene glycol dimethacrylate (EDGMA) crosslinkers that function to form bonds that connect one polymer chain to other polymers, and 2,2-`azoisobutironitril (AIBN) crosslinkers that function to form bonds that connect one polymer chain with other polymers, and 2,2-

`azoisobutironitril (AIBN) initiators that function to increase the speed of the polymerization reaction, in chloroform solvents.

The reactions that occur between mold molecules, functional monomers and initiators are based on non-covalent interactions that are often used because they have several advantages for later stages. In MIP synthesis, the mold molecules used are small organic molecules. Acrylamide used as a functional monomer has better polymer results because it is responsible for the interaction of bonds in the polymer bonding side. EGDMA which is used as a crosslinker function in controlling the morphology of the polymer matrix, stabilizing the polymer bonding side and providing mechanical stability with the polymer matrix. AIBN which is used as an initiator is one of the azo compounds that can form free radicals at 60° C at the time of polymerization. Chloroform is used as a porogen solvent because caffeine solubility is very soluble in chloroform [17, 18].

Furthermore, the flow of nitrogen gas for 5 minutes and then sonicated aims to remove oxygen gas retained in the reaction bottle because in the presence of oxygen there will be a slowing of polymerization so that peroxide is formed resulting from the reaction between oxygen gas and free radicals [18].

The reaction bottle containing clear synthesis is heated at 60° C for 8 hours which is observed every 1 hour. The NIP synthesis was made with the same amount and treatment as MIP synthesis, but in the synthesis of NIP, the addition of caffeine as a molding molecule was not carried out. The results of the synthesis of MIP and NIP in the form of crystals, obtained at MIP = 9.21 grams and NIP = 7.91 grams. Next, MIP and NIP are crushed slowly and sifted between mesh 40 and mesh 60 to produce more uniform crystals [13, 21].

Release of the mold molecule is carried out by the sonication method. As much as 1.5 grams of MIP are dissolved in 10 mL chloroform sonicated for 30 minutes to release caffeine in the polymer matrix so that it will produce an empty cavity in MIP that can bind caffeine back. Furthermore, filtering is carried out to separate the filtrate and extractant. The residue is then dried to produce decaffeinated dry MIP crystals. Caffeine released from MIP is indicated by the results of concentration measurements below the detection limit and the results of infrared spectrum analysis on MIP and NIP. repeated until the extractant concentration was below the detection limit of 1.173 ppm.

FTIR characterization was carried out on caffeine, NIP before extraction, MIP without template and MIP before extraction, to find out that the results of the polymerization process were as expected. Caffeine molecules extracted through the release of molecules have been released completely to produce MIP with a space that is able to recognize specific analytes [18]. Caffeine as a template is a group of amines, with the emergence of NH stretching uptake at wavenumbers 3600-3200 cm^{-1} [15]. The acrylamide compound has a C = group O, CH, -CH2, -NH2 and CN. Based on the FTIR test results, it can be seen that in the MIP before extraction, MIP without a template and NIP has a peak absorption of C = O which appears in the range of wave numbers 1680-1630 cm^{-1} [19].

MIP spectra with templates and MIP without templates show that polymers have formed which are indicated by the absorption at 1800-1000 cm^{-1} wavenumbers. The bonds formed are hydrogen bonds from the presence of acrylamide between the carbonyl group of acrylamide and the proton donor from caffeine, the oxygen atom in the carbonyl group as the hydrogen bond acceptor, while the amen group as the hydrogen bond donor. At the wave number 2400-2000 cm^{-1}, the polymer has been formed which is the result of excitation from the ground state to a higher energy state [19].

In NIP and MIP without a template, there was no absorption peak found by N-H functional groups, but in MIP before extraction, there was found absorption of functional groups at wavenumbers 3049.99 cm^{-1}. MIP without template still shows absorption of amine groups at wave number 1457.74 cm^{-1}, meaning that caffeine in MIP has not been extracted entirely.

The binding process that was observed from 20 mg MIP without template and NIP was dissolved in 5 mL chloroform each with concentrations of 9, 11, 13, 15, 17, and 19 ppm. With the batch method, all 6 MIP and NIP concentrations were incubated for 24 hours with the aim that the caffeine interactions occur with MIP, then filtered, measured using UV-VIS Spectrophotometry and the concentration calculated [13, 21].

This adsorption is the adsorption isotherm that is commonly used, namely the Langmuir and Freundlich adsorption models. The Langmuir model occurs on homogeneous or monolayer surfaces, while the Freundlich model occurs on multilayer surfaces which explain the heterogeneity of the surface to produce different energy [20].

Quadratic and integral equations are used to calculate the area under the MIP and NIP curves to obtain the Imprinting Factor (IF). IF value obtained at 1.261 indicates that MIP adsorbs better than NIP or MIP without a template has been able to bind caffeine [20].

From the isotherm adsorption curve between Langmuir and Freundlich, the equation used is the equation with the largest R^2 value that follows the Freundlich isotherm model. This model explains that the MIP that has been created has a heterogeneous surface and that each printed molecule has different absorption potential. From the 6 concentration of MIP adsorption results without template and NIP the desorption process was carried out with the addition of 5 mL chloroform, sonicated 5 minutes then filtered. The resulting filtrate was measured using UV-VIS Spectrophotometry [19].

The results of the analysis of the determination of desorption capacity with linear equations for MIP are $y = 0.0907x + 4.883$ with $R^2 = 0.9692$ resulting in a recovery of 46.28%, while for NIP is $y = 0.0617x + 5.1174$ with $R^2 = 0.9773$ resulted in a recovery of 45.14%. This shows that chloroform can adsorb caffeine because chloroform is a porogen solvent used in the manufacture of MIP.

The ability of MIP in MISPE is better than NIP because MIP has a cavity that can bind caffeine back. This cavity interacts with sorbent while NIP does not interact with diluted theophylline, 1.31% of theophylline is absorbed, while in

caffeine 76.35%. This shows that MIP with caffeine mold molecules is more selective than theophylline.

MIP selectivity testing is carried out on caffeine and samples that have a group and use almost the same as caffeine, theophylline. Based on the results of testing with 50 mg MIP without templates, MISPE and NISPE testing are done by extracting analgesic drug samples and energy drinks containing caffeine with chloroform, then added to each 50 mg MIP without templates and NIP that have been inserted into the MISPE cartridge modification column and NISPE. Based on the test results obtained 82.01% for MISPE analgesic drugs (in 35 mg caffeine), 0.65% for NISPE analgesic drugs (in 35 mg caffeine), 84.02% for MISPE energy drinks (in 50 mg caffeine), and 4.06% for NISPE energy drinks (in 50 mg caffeine).

V. CONCLUSION

The application of the method used in the synthesis of MIP and NIP produces the desired crystalline polymer ie the MIP produced has better selectivity compared to NIP. The results of caffeine testing in MIP and NIP using UV-VIS Spectrophotometry obtained an IF value of = 1.261. The MIP and NIP isotherm adsorption mechanism follows the Freundlich model because it is seen from the greatest R^2 value. The selectivity test results were 1.31% which were absorbed in MIP by theophylline, while in caffeine it was 76.35%. So that MIP is more selective about caffeine compared to theophylline.

The percentage value of recovery for the application of MISPE in samples of analgesic drugs and energy drinks were 82.01% and 84.02%, respectively. The application of NISPE in analgesic drugs and energy drinks samples were 0.65% and 4.06%, respectively. The results showed that the solid phase sorbent extraction by molecular imprinting technique for selective caffeine extraction could be made by the polymerization of the broth method. Acrylamide monomers are functional monomers that can be used for the development of selective phase solid sorbents for caffeine compounds.

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